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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re application of
Harrington, J. et al.
Application No. 09/276,820
Filed March 26, 1999

For: Compositions and Methods
For Non-targeted Activation
of Endogenous Genes

Art Unit: 1633
Examiner: R. Shukla
Atty Docket: 5817-7

Declaration Under 37 C.F.R. § 1.132

3/27/00
RPS
Assistant Commissioner of
Patents and Trademarks
Washington, DC 20231

Sir:

The undersigned, John J. Harrington, declares and states:

1. I am an inventor of the above-captioned patent application, U.S. Application No. 09/276,820, filed March 26, 1999, entitled "Compositions and Methods for Non-targeted Activation of Endogenous Genes." I am the subject of the attached Curriculum Vitae and author of the publications shown on the list attached thereto. On the basis of the information and facts contained in these documents I submit that I am an expert in the field of non-homologous recombination, eukaryotic gene expression and gene cloning and am qualified to speak on the skill and knowledge of the person of ordinary skill in these fields.

2. I have read and understand the subject matter of the above-captioned application.

I have read and understand the Office Action dated February 1, 2000, rejecting claims 73, 77-80, 82, 85-93, 98-106, 108-123, 128-132, 180-183, 223-224, and 226. It is my opinion, based on the scientific evidence and reasoning set forth below, that the methods and compositions that are the subject of the rejected claims (indicated above) could have been made and used by the person of ordinary skill in the art, as claimed, as of the filing date of September 26, 1997, by routine and ordinary experimentation, using the '820 specification as a guide.

3. It is my opinion that a broad range of genes, including those claimed, could have been over-expressed *in vivo* and *in vitro* (as of September 26, 1997), using the compositions and methods as disclosed in the '820 specification, using routine and ordinary experimentation. My opinion is based on the following results.

4. The Examiner has raised a number of issues and questions that I would like to address below, in sequence. First, however, I would like to address a statement made by the Examiner on page 8, second full paragraph, of the Office Action.

The Examiner contends that the "claimed invention is limited by the fact that it does not use a sequence that will have homology to a known gene, in other words integration of the vectors are not by homologous method but by random integration and non-homologous recombination."

The randomness of the present invention is not a limitation, but instead is a powerful asset. I point out that random integration allows all genes to be activated, allowing comprehensive protein expression libraries to be created. Homologous recombination, on the other hand, allows activation of only genes that have been previously identified, cloned, and characterized by others. Furthermore, the present invention, as described in the application and discussed below, can be used to discover and isolate novel genes that are difficult or impossible to isolate using other approaches. Gene activation by homologous recombination cannot be used to discover genes. Thus, there are clear advantages to the use of activation of genes using non-homologous recombination.

5. I now address each of the issues and questions raised by the Examiner on pages 3-5 of the Office Action. I will first cite the Examiner's statement and then address the statement.

A. "While the specification has provided diagrams of vectors that comprise claimed sequence elements and protocols to make libraries, PCR and other techniques of making cell lines, libraries etc., there is no evidence that all of the claimed endogenous genes would have been activated by the claimed methods and would have yielded increased production of proteins from the endogenous genes listed." Paragraph spanning pages 3-4.

First, I point out that the exemplary material provides evidence for the activation of any given gene, including the listed genes. In the exemplary material, we (the Applicants) have demonstrated activation of integral membrane proteins in an experimental protocol specifically designed to identify

such proteins. The Examiner is directed to page 74 of the Applicants' specification for the general rationale. The text is cited below, for the Examiner's convenience.

To identify genes that encode integral membrane proteins, vectors integrated into the genome of cells will comprise a regulatory sequence linked to an exonic sequence containing a start codon, a signal sequence, and an epitope tag, followed by an unpaired splice donor site. Upon integration and activation of an endogenous gene, a chimeric protein is produced containing the signal peptide and epitope tag from the vector fused to the protein encoded by the downstream exons of the endogenous gene. This chimeric protein, by virtue of the presence of the vector encoded signal peptide, is directed to the secretory pathway where translation of the protein is completed and the protein is secreted. If, however, the activated endogenous gene encodes an integral membrane protein, and the transmembrane region of that gene is encoded by exons located 3' of the vector integration site, then the chimeric protein will go to the cell surface, and the epitope tag will be displayed on the cell surface. Using known methods of cell isolation (for example, flow cytometric sorting, magnetic bead cell sorting, immunoadsorption, or other methods that will be familiar to one of ordinary skill in the art), antibodies to the epitope tag can then be used to isolate the cells from the population that display the epitope tag and have activated an integral membrane encoding gene. These cells can then be used to study the function of the membrane protein. Alternatively, the activated gene may then be isolated

from these cells using any art-known method, e.g., through hybridization with a DNA probe specific to the vector-encoded exon to screen a cDNA library produced from these cells, or using the genetic constructs described herein.

The epitope tag encoded by the vector exon may be a short peptide capable of binding to an antibody, a short peptide capable of binding to a substance (e.g., poly histidine/divalent metal ion supports, maltose binding protein/maltose supports, glutathione S-transferase/glutathione support), or an extracellular domain (lacking a transmembrane domain) from an integral membrane protein for which an antibody or ligand exists. It will be understood, however, that other types of epitope tags that are familiar to one of ordinary skill in the art may be used equivalently in accordance with the invention.

In the exemplary material, pRIG8R1-CD2 (Figure 5A) is used as the activation vector. In this vector, the exon encodes a signal peptide linked to the CD2 extracellular domain. Therefore, activated proteins are secreted but integral membrane proteins will be embedded in the cell membrane where they can then be detected by means of the CD2 extracellular sequence (on the cell surface) by anti-CD2 antibody.

This assay shows that a specific class of protein can be activated and isolated, as predicted by Applicants' specification. Since this class can be activated as predicted, it is reasonable to predict that the other classes will be activated as well. Using the specification and general knowledge (as of the filing date) available to the person of ordinary skill in the art, we have, in fact, demonstrated activation

of genes disclosed in the specification and claims of the application. The activation of these genes demonstrates a high probability that the person of ordinary skill in the art would have been able to activate any given gene, including the claimed genes. To activate expression of these genes, we (the Applicants) created gene activation libraries using the methods set forth in the specification and in the Examples. These libraries were screened using rtPCR to detect activation of a number of genes listed in claims 90-97. Specifically, we tested these libraries for activation of erythropoietin (Epo), growth hormone (hGH), GCSF, glucocerebrosidase, and Factor VIII. None of these genes were expressed in the parental cells used to create the gene activation library. As a result of the PCR screening, we have identified clones of cells expressing each of these genes. Since none of these genes were expressed in the parental cell line used to create the activation library, we have therefore shown that each of these genes was activated and expressed at levels higher than those found in the parent cell line.

In addition to the above experiments, we have created gene activation libraries using methods described in the specification. To date, an estimated 20,000 individual activated genes have been isolated from these libraries, a number that continues to increase. Approximately 50% of these genes are novel. More relevant to the current discussion is the fact that many of the genes that we have activated and isolated are listed in the application on pages 43-44 and in claims 90-97. For example, we have activated expression of interleukin-6, interleukin-10, macrophage colony-stimulating factor (M-CSF), bone derived growth factor, transforming growth factor-beta, transforming growth factor beta 3, transforming growth factor beta-2, thyroid stimulating hormone beta (TSH-b), antithrombin III, glucagon, protein kinase C, nerve growth factor, insulin like growth factor 4, lactoferrin, alpha glucosidase, and fibroblast growth factor 12B. We have also activated and isolated a number of CD

genes, including CD5L, CD24, CD26, CD36, CD53, and CD59. We have also activated many different growth factors and cytokines (referred to generically in the specification on page 44, line 6) including, but not limited to, interleukin-1B, interleukin 15, interleukin 18, bone morphogenic protein 5, FHF-1, and glial growth factor. In short, we have activated (and isolated) all of the major classes of genes including, but not limited to, growth factors, cytokines, transcription factors, enzymes, structural proteins (such as extracellular matrix proteins and cytoskeletal proteins), receptors, secreted factors, ion channels, G-protein coupled receptors, and many other types of proteins.

To further demonstrate that the disclosed invention can be used to over-express proteins, we characterized cell lines expressing two proteins, Epo and hGH, two proteins exemplified in the specification on page 43 and in claims 90-97. To create cell lines expressing these proteins, a vector called pRIG-1 described, *inter alia*, in Example 1 and Figure 4, and using the transfection method described in detail in Example 1, we created a gene activation library in a human cell line. The library was then screened using an ELISA (a screening approach set forth on page 65 of the specification) to screen for expression of human growth hormone or erythropoietin. Library pools containing Epo or growth hormone were identified, and using limiting dilution and sib selection (two procedures set forth on page 65 lines 14-16 and page 69 lines 8-13) of the specification, individual clones expressing either Epo or hGH, as the case may be, were shown to be producing large quantities of protein (up to 1 ug/ 10^6 cells/day for hGH and up to 6000 mUnits/ 10^6 cells/day for Epo). To confirm that pRIG-1 had integrated in the vicinity of these genes in each of the respective clones, rtPCR was performed with a forward primer to pRIG-1 activation exon and a gene specific reverse primer. The presence of activated transcripts containing the activation exon from pRIG-1 demonstrates that the increased expression of

Epo and hGH is the result of integration of pRIG-1 into or upstream of the respective genes, and transcriptional activation from the CMV promoter present on the integrated vector.

Thus, we have demonstrated that the disclosed invention can be used to express the genes listed on pages 43-44 and in claims 90-97 in the application. This experimentation was done using the specification as a guide and with routine and ordinary experimentation available to the person of ordinary skill in the art at the time that the application was filed.

B. “On page 135 of the specification, lines 9-30 continued on page 136 lines 1-3 disclose the results of an experiment of activating expression of transmembrane proteins. The specification discloses that in one screening, of eight isolated activated genes, 4 encoded known integral membrane protein genes whereas 6 encoded novel genes. However, none of the isolated genes encoded a growth factor, cytokine or hormones or other proteins listed, for example in claim 90. In yet another example, the specification discloses that of 11 genes isolated, one had sequence homologous to a partially sequenced integral membrane protein gene whereas 9 were novel genes. Again, even this screening did not result in the identification of any of the genes listed, for example, in claim 90.” Paragraph spanning pages 3-4.

The experiments referenced above, and described on page 135 of the specification, relate to one embodiment of the invention and focus on discovery of genes encoding integral membrane proteins. This embodiment of the invention has been discussed above. The method selects for cells in which integral membrane proteins are activated. See 5A above. The methods actually are designed for removing cells that have not activated genes that encode integral membrane proteins (since such cells

will not be positive for anti-CD2 antigen on the cell surface). Since the cytokines, growth factors, and enzymes listed in claim 90 are not integral membrane proteins, we would not expect to see them in this group of genes.

With respect to the receptors and ion channels listed generically in claim 90, I point out that the 21 genes referenced by the Examiner (*i.e.*, 10 and 11 isolated genes) represent only a small fraction of the estimated 140,000 genes in the human genome (only ~ .015% of the genes in the genome). Therefore, the probability of observing, in a sample of 21 genes selected at random, the receptor for any of the ~70 proteins listed in claim 90 is very small. Thus, in the cited example, for this further reason, we would not expect to see a specific gene listed in claim 90 due to the small sample size.

I point out, however, that the data in the referenced example shows very clearly that known and novel integral membrane protein encoding genes can be identified using this embodiment of invention.

C. “If an artisan had to target a known gene, for example in the claimed list, what is the probability that the candidate gene will be activated. If one was targeting insulin receptor or GM-CSF or M-CSF or so on, based on the results disclosed in the specification, one has to conclude that the probability will be zero because none of the listed genes were activated in the provided examples.”

Paragraph spanning pages 3-4.

The probability of activating expression of a given gene, such as GM-CSF, M-CSF, is not zero. As discussed above in 5A and 5B, the cells in the exemplary material were selected on the basis of a

specific type of gene activation. This limited the type of gene detected to genes whose expression product would be embedded in the cell membrane. Further, I point out that the small sample of genes disclosed in the examples was meant to be illustrative, not comprehensive. As discussed above, such a small sample size, further limited by a specific screening assay, would not be expected to include many genes on the list. Furthermore, the list of genes disclosed in the specification on pages 43 and 44, as stated above, was in no way meant to be limiting. As discussed in 5A, however, we have activated many of the genes on the list.

In addition to activating the listed genes, we have activated a large number of genes that were not listed in the specification. In fact, we have activated (and isolated) an estimated 20,000 unique human genes. This number is increasing each day. The vast majority of these genes were not even listed in the specification.

We have also shown that we can activate gene expression from a variety of selected genes not listed. That is, when we screened our libraries for specific genes of interest, we found that invariably we obtained activation of that gene. For example, we have screened activation libraries for expression of a variety of receptors such as the alpha-3, alpha-4, alpha-7, and beta-2 subunits of the nicotinic acetylcholine receptors, and glucagon like peptide-1 receptor, to name a few. For each of these genes,

we identified activated cells expressing the previously silent gene. Again, these examples are not meant to be limiting.

Thus, the probability of activating expression of a given gene, whether listed or not in the specification, is not zero. To summarize, we have, in fact, activated expression of an estimated 20,000 genes. We have also been able to activate gene expression from every gene tested to date, including many genes on the list and many genes not present on the list.

I would also like to clarify that we do not target specific genes. The disclosed invention allows random or semi-random gene activation libraries to be created and screened. Within these libraries, many genes have been activated and it is simply a matter of screening the library with a suitable assay for the protein or gene of interest. Thus, while the invention can be used to isolate cells expressing particular genes, such as those listed in the specification and many others, the invention does not involve targeting those genes specifically.

D. “Even in the case of a transmembrane protein, while the specification discloses that four of the identified genes were transmembrane genes, there is no disclosure or evidence as to how many fold the expression of the isolated gene was increased or in fact whether there was any increase in the expression. Furthermore, did the isolated cell express full length transmembrane protein, was the protein functional, and if not what would have been the use of providing such cells to an animal or to a human.”

First, I again point out that the invention is not limited to cells expressing transmembrane proteins. For example, claims 90-97 are directed to cells expressing many different secreted proteins. Also, a number of claims are directed to methods for expressing any type of protein or expression

product (see, for example, claim 77). Again, the specification enables isolation and use of cells expressing any endogenous gene, including but not limited to secreted factors, transmembrane proteins, and intracellular proteins. Support for activation of all types of genes may be found, *inter alia*, on pages 43, 44, and 59 of the specification.

I would also like to point out, however, that claims are directed to over-expression or activation. Even two-fold increase would constitute activation. In the exemplary experiments, no CD2 antigen was present in the parental cells. The presence of the antigen shows over-expression of the membrane proteins in the selected cells. Nevertheless, as discussed below, activation has been found to be many-fold.

We have demonstrated that virtually any type of gene can be activated by the methods of the present invention. We have also isolated cells from gene activation libraries, confirmed the mechanism of activation, and shown that expression of the proteins encoded by these genes had increased dramatically prior to activation. Neither Epo nor hGH expression could be detected in the parent cell by a very sensitive technique, rtPCR. Following gene activation, however, expression of the RNA transcript and protein of interest could be easily detected. Furthermore, protein expression was found to have increased in these cells by an estimated 100 to 1000-fold, as determined by ELISA. Likewise, gene activation libraries were screened for expression of several transmembrane protein encoding genes, including several alpha and beta sub-units of the nicotinic acetylcholine receptors and the GLP-1 receptor. In each of these cases, the gene was not expressed in the parent cell, and was expressed at high

levels in the activated cell, as demonstrated by semi-quantitative PCR. The activated and expressed genes were found to encode full length proteins in these cells.

To further demonstrate the high level of expression of proteins activated by the present invention, cells expressing Epo and hGH were subjected to increasing concentrations of methotrexate. Following several rounds of gene amplification, expression levels were found to have further increased by several orders of magnitude.

The secreted proteins produced by these cells were found to be full length and properly processed as determined by SDS-PAGE and western blotting. hGH was confirmed to be full length and properly processed. In addition, hGH has been sequenced at the N-terminus and was found to have the proper N-terminal amino acid sequence.

Both Epo and hGH produced above displayed immunoactivity with monoclonal antibodies, correct glycosylation patterns, and correct molecular weight. These characteristics have been shown to be possessed by active forms of Epo and hGH, thereby showing that proteins produced by the methods of the present invention have the characteristics of naturally produced proteins.

Additional genes have also been activated and characterized and the above examples are not meant to be limiting.

Finally, I would like to point out that, as disclosed in the specification, the goal of gene activation is not always to produce full-length protein. In several embodiments of the invention, such as are disclosed on page 43 lines 6-13 and page 44 lines 20-26, it is desirable to produce a truncated protein. In some cases, the truncated protein will retain one or more of its biological activities. In other cases, the activated protein will not retain any of its biological activities. Both biologically active and inactive proteins are useful as antigens. In other instances, truncated proteins may create dominant negative or dominant positive phenotypes allowing identification of novel genes or characterization of biological processes. Thus, while production of full-length proteins may be important for some applications, in other applications, production of truncated proteins may be desirable.

In other embodiments of the invention, the activation vector may be designed to prevent protein expression from the activated gene. For example, pRIG-20 disclosed in Figures 31A-31C, is designed to prevent protein expression while still producing a “tagged” transcript useful for isolating cDNA clones from novel genes.

E. “When these cells were administered in an animal, would the expression of the transmembrane protein have been the same or more or would have ceased? Compared to the in vitro method, would the expression of the gene when the cells are administered to a human or animal have been high enough to detect the expressed protein. The specification does not provide any guidance to any of these problems as to how an artisan of skill would have been able to make and practice the invention as claimed.”

As demonstrated above, the present invention has been used to create cells expressing large quantities of native, properly processed protein from a variety of endogenous genes. Furthermore, the specification describes a variety of methods for regulating gene expression of activated genes through the use of cellular and viral promoters, constitutive and inducible promoters, enhancers, and locus control regions. In this respect, expression levels and transcriptional regulation of activated genes is comparable to expression of cloned cDNA fragments. Thus, cells expressing genes activated by the present invention can be used, *in vitro* and *in vivo*, the same way as cells expressing cloned cDNA fragments.

A variety of data demonstrate that the cells of the invention can be used *in vivo* to express genes. First, transgenic animals expressing heterologous genes have been created and studied. See, for example, Wang *et al.* (1997) *Proc. Nat'l Acad. Sci. U.S.A.* 94: 212-226. These animals have been found to express the desired protein constitutively or in a tissue specific fashion, depending on what type of transcriptional regulatory sequences are used. As discussed above, the present invention discloses a variety of tissue specific, constitutive, and inducible promoters and enhancers useful for practicing the invention. The specification also discloses use of a variety of regulatory elements that have been shown to be useful for obtaining long term, tissue specific gene expression *in vivo*. Examples of these elements, disclosed on page 46 lines 8-11, include locus control regions, scaffold attachment regions, and matrix attachment sites.

Second, it is well known that cells can be modified *in vitro* to express a transgene and introduced into animals by adoptive transfer. In the animal, the cells continue to express the transgene at levels high enough to observe the effect on various biological processes. See, for example, Mathisen *et al.* (1997) *J.*

Exp. Med. 186: 159-164; Chen *et al.* (1995) *J. Neurosci.* 15: 2819-2825. Again, cells of the present invention can be engineered to have the same expression characteristics as the cells in these previous studies.

Thus, in each of these *in vivo* approaches (production of transgenic animals, adoptive transfer [*ex vivo* gene therapy], and *in vivo* gene therapy), the cells of the present invention can be used to express genes, both full length and truncated, *in vivo*.

F. "Arguments presented above and the examples disclosed in the specification would indicate that the claimed method results in random activation and there is no way of finding out which gene has been activated. If so, how can an artisan use this method to activated any endogenous gene, prepare a library, isolate cells and use them for administration to an animal if it was not known what gene was activated. Likewise, how can the method be used for *in vivo* activation of endogenous genes without knowing which gene will be activated. Furthermore, how can a library be screened if an artisan did not know which genes were activated because without this information for what gene will an artisan screen for? In conclusion, how can a particular endogenous gene be targeted by a random integration of any of the given vectors. In conclusion, the specification does not provide any evidence as to whether the claimed method would have activated any and all the listed endogenous genes."

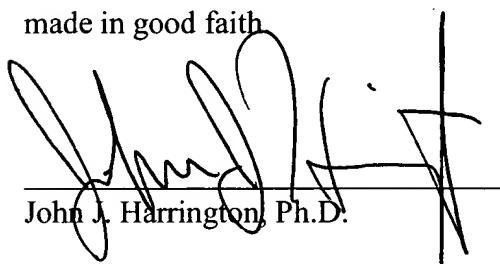
The specification does, in fact, teach how to make and screen activation libraries and how to isolate activated genes. For example, on pages 65-68, the specification describes methods for identifying cells that have activated expression of specific genes, known or previously undiscovered. Disclosed methods for identifying cells expressing known genes include, but are not limited to, ELISA,

ELISA spot, Fluorescence Activated Cell Sorting, magnetic beads cell separation, rtPCR, bioassays, and enzymatic assays. Disclosed methods for identifying cells expressing previously unknown proteins or activities include phenotypic screens, such as cell proliferation, growth factor independent growth, colony formation, cellular differentiation, anchorage independent growth, activation of cellular factors, gain or loss of cell-cell adhesion, migration, cellular activation (e.g. resting versus activated T cells). In other words, any gene that upon activation gives an observable phenotype can be detected. Once detected, using methods disclosed in the specification (such as on page 69, lines 8-13 and in examples 5 and 8) or other methods known in the art, the cell of interest can be isolated. Alternatively, the cell of interest may be left unpurified, for example, in a pool of library clones. In either case, the activated gene(s) can be isolated using methods described in the specification (such as in examples 5 and 8) or using other methods well known in the art such as 3' RACE (see methods used in Datson *et al.* (1994) *Nucl. Acids Research* 22: 4148-4153 and in Datson *et al.* (1996) *Nucleic Acids Research* 24:1105-1111) or using hybridization (see methods in Shen-Ong *et al.* (1986) *Molecular and Cellular Biology* 6:380-392).

Isolation of activated genes, known or unknown, is described through out the specification. For example, we have isolated over 20,000 genes (10,415 of which are novel) using the methods described in the specification and in examples 5 and 8 or by using standard molecular biology techniques such as 3' RACE. Each of these genes was isolated without any knowledge of their nucleic acid sequence, expression pattern, chromosome location, or gene structure. Thus, any gene can be isolated regardless of its sequence, structure, chromosome location, or normal expression pattern and regardless of whether it is known or previously undiscovered, characterized or uncharacterized.

6. It is, accordingly, my opinion that the '820 specification provides sufficient guidance so that the person of ordinary skill in the art could have practiced the invention as claimed, at the time of filing (September 26, 1997), using routine and ordinary experimentation.

7. I further declare that all statements made herein as my own knowledge are based on a good faith belief that they are true and any statements made on information and belief are made in good faith



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April 20, 2000

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EDUCATION

- 1994-1998 Case Western Reserve University, Cleveland, OH
Post-doctoral Research in Human Molecular Genetics
- 1989-1994 Stanford University, Stanford, CA
Ph.D. in Cancer Biology
- 1985-1989: University of California, San Diego, San Diego, CA
B.A. in Biochemistry and Cell Biology (Cum Laude)

RESEARCH EXPERTISE

- Protein Biochemistry Protein purification, gene overexpression, assay design, enzyme kinetics, protein structure-function analysis, identification of novel enzymatic activities
- Molecular Biology Molecular gene cloning, PCR, cDNA and genomic library construction, nucleic acid enzymology, vector construction, Southern and Northern Blot analysis

RESEARCH AND MANAGEMENT EXPERIENCE

- 1995-present Executive Vice President, Chief Scientific Officer, and Director, Athersys, Inc. Responsible for directing all research and development activities at the Company, including development of the Company's proprietary technology platforms. Along with the Board of Directors, responsible for oversight on all matters related to Company operations, including budgetary, legal, and strategic issues.
- 1994-1998 Post-doctoral Research Associate, Case Western Reserve University Constructed Human Artificial Chromosomes. Developed assays for characterization of human artificial chromosomes and the genetic elements required for chromosome function.

- 1989-1994 Graduate Research Associate, Stanford University.
Dissertation Research: Purification, characterization, and molecular cloning of the FEN-1 family of structure-specific endonucleases.
Additional Research: Developed cellular and cell-free assays for V(D)J recombination.
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- 1988-1989 Research Assistant, Scripps Clinic and Research Foundation.
Purification and characterization of Protein C Inhibitor from human plasma.
Principal Investigator: Dr. John Griffin

MEETINGS AND ABSTRACTS

List available upon request

PUBLICATIONS

Harrington, J. J., C. L. Hsieh, J. Gerton, G. Bosma, M. R. Lieber (1992). Analysis of the Defect in DNA End Joining in the Murine scid Mutation. *Mol. Cell. Biol.* 12(10): 4758-4768.

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Hiraoka, L., **J. J. Harrington**, D. S. Gerhard, M. R. Lieber, and C. L. Hsieh (1995). Sequence of Human FEN-1, a Structure-Specific Endonuclease, FEN-1, and Chromosomal Localization in Mouse and Human. *Genomics* 25: 220-225.

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X. Li, J. Li, **J. J. Harrington**, M. R. Lieber, P. M. J. Burgers (1995). Lagging Strand DNA Synthesis at the Eukaryotic Replication Fork Involves Binding and Stimulation of FEN-1 by PCNA. *J. Biol. Chem.* 270(38): 22109-22112.

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PATENTS

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U.S. patent # 5,874,283. Mammalian flap-specific endonuclease (1999); **Harrington, J. J.**, Hsieh; C.L., M.R. Lieber.

U.S. patent # 5,695,967. Method for stably cloning large repeating units of DNA (1997); Van Bokkelen; G.B., **Harrington; J. J.**, and H.F. Willard.

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